This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 February 2002 (28.02.2002)

PCT

(10) International Publication Number
WO 02/15924 A1

(51) International Patent Classification[7]: A61K 38/17, A61P 35/00

(21) International Application Number: PCT/GB01/03682

(22) International Filing Date: 17 August 2001 (17.08.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0020504.7 18 August 2000 (18.08.2000) GB

(71) Applicant *(for all designated States except US)*: **THE UNIVERSITY OF BRISTOL** [GB/GB]; Senate House, Tyndall Avenue, Bristol BS8 1TH (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ALLEN, Shelley, Jane** [GB/GB]; Molecular Neurobiology Unit, Department of Medicine, University of Bristol BS2 8HW (GB). **DAWBARN, David** [GB/GB]; Molecular Neurobiology Unit, Department of Medicine, University of Bristol BS2 8HW (GB).

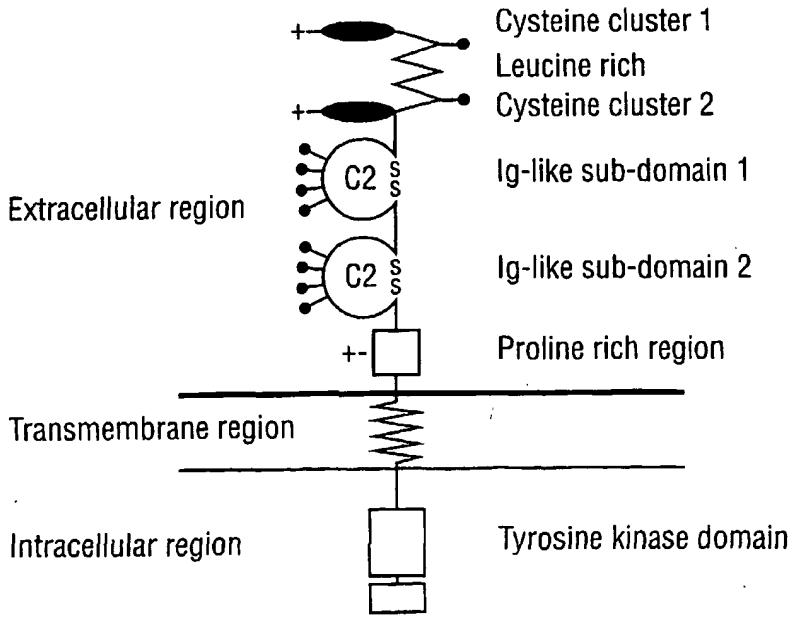
(74) Agents: **TOMBLING, Adrian, George** et al.; Withers & Rogers, Golding House, 2 Hays Lane, London SE1 2HW (GB).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: PHARMACEUTICAL COMPOSTION COMPRISING TRKAIG2 FOR USE IN THE PREVENTION AND/OR TREATMENT OF CANCER



(57) Abstract: This invention relates to the treatment of cancer and is particularly, though not exclusively concerned with the treatment of pancreatic cancer. In particular the invention related to the use of TrkAlg2 in the preparation of a medicament for the treatment and/or prevention of cancer in a patient.

WO 02/15924 A1

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

PHARMACEUTICAL COMPOSITION COMPRISING TRKAIG2 FOR USE IN THE PREVENTION AND/OR TREATMENT OF CANCER

The present invention relates to the treatment of cancers, in particular, the present invention relates to the treatment of pancreatic cancer.

Nerve growth factor (NGF) and its high-affinity tyrosine kinase receptor A (TrkA) are generally considered to be involved in neural development and survival and growth of central and peripheral nerves.

NGF may be isolated from various sources, most particularly from male mice salivary glands. It may be isolated first as 7S NGF, named for its sedimentation coefficient, which is a complex of β-NGF and γNGF. 2.5S NGF may be obtained from this. 2.5S NGF is known to be responsible for the neurotrophic biological activity of the complex. 2.5S NGF is βNGF but often partially proteolysed at the amino and carboxy termini. NGF is one member of a family of related proteins, the neurotrophins. The other members include for example BDNF, NT-3 and NT-4. All of the neurotrophins bind to a common receptor p75NGFR. Each also binds to one of a homologous family of tyrosine kinase receptors: NGF binds to TrkA, BDNF and NT-4 bind to TrkB, and NT-3 binds to TrkC. NT-3 can also bind TrkA and TrkB with reduced affinity.

Recently two groups have shown that the Ig-like domains of the Trk receptors play important roles in the binding of neurotrophin ligands and receptor activation. Perez P. *et al* (Molecular and Cellular Neuroscience 6: 97-105 (1995)) concluded that both of the Ig-like domains are important for the binding of NGF to TrkA. The co-crystal structure of the NGF homodimer and TrkAIg2 has now been solved (Weismann *et al.* Nature **401**, p184-188 (1999)).

TrkA and isolated domains thereof are further described in WO 99/53055, the disclosure of which is incorporated by reference. The accompanying Figure 1 illustrates its structure schematically. The filled circles represent consensus glycosylation sites. TrkAIg2 is defined as including Ig-like sub-domain 2 and the proline rich region. The sequence of TrkAIg2 is shown in Figure 2 which shows the nucleotide sequence and derived amino

acid sequence of TrkAIg2 with 6 x His tag. Sequence from human TrkA is in bold, 6 amino acid insert variant is underlined. This sequence includes the human TrkA sequence (amino acids 22 to 150) and a flanking sequence from the pET15b vector (amino acids 1 to 21) which also codes for an N-terminal 6 x His tag. The vector sequence (codons 452 to 468, Figure 2) also provides for a stop codon.

The putative extracellular domain of human TrkA is taken to be either 375 or 381 amino acids long depending on whether the 6 amino acid insert VSFSPV is present. The inventors have recently shown that a protein comprising the two immunoglobulin-like domains and proline-rich region (shown in Fig. 1 as Ig-like subdomain 1, Ig-like subdomain 2 and proline rich region) alone are able to bind NGF with a similar affinity to that of the complete extracellular domain (Holden, P. H. *et al* (1997) Biotechnology **15**: 668-672). This region is defined here as TrkAIg1,2. In addition, the inventors have found that an even smaller domain of TrkA referred to as TrkAIg2 (shown in Fig. 2 as amino acids 22 to 150) is able to bind NGF with a similar affinity to the complete extracellular domain or the TrkAIg1,2 region and is thus responsible primarily for its binding properties. TrkAIg2 is defined herein as including the TrkAIg-like sub-domain2 together with the proline rich region, spanning amino acids 22 to 150 as defined in Figure 2 and may also contain amino acids 1 to 21, and may or may not include the six amino acid insert VSFSPV as shown as amino acids 130 to 135 also in Figure 2.

The pancreas is a gland which makes pancreatic enzymes for digestion of food. These are released into ducts which pass into the bile duct and into the duodenum. The pancreas also produces several hormones, including insulin.

Cancer of the pancreas is the fifth highest cause of cancer-related death in the Western world. It accounts for 2% of newly diagnosed cancers in the US each year, but 5% of all cancer deaths, and has the poorest survival rate of all of the major malignancies. Over 26,000 people in the US present with cancer of the pancreas each year. Men have a higher incidence of pancreatic cancer and resulting mortality rate than women. Those of Afro-Caribbean descent have incidence and mortality rates that are about 50% higher than

the rates for Caucasians, whilst the rates for Hispanics and the Asian-American groups are generally lower.

Most pancreatic cancers are adenocarcinomas arising from the ducts. The disease is often advanced by the time symptoms present, with less than 5% of sufferers surviving after 5 years, as successful treatment is rare. 2% of pancreatic cancers are islet cell cancer (i.e. cancers of the islets of Langerhans that produce insulin and other hormones). These have a better prognosis. As pancreatic cancer grows, the tumour may invade organs that surround the pancreas, such as the stomach or small intestine. Pancreatic cancer cells may also metastasise and spread to other parts of the body, often forming new tumours in lymph nodes, the liver, and sometimes in the lungs or bones.

When symptoms appear, they depend on the location and size of the tumour. For example, if the tumour blocks the common bile duct so that bile cannot pass into the intestines, the skin and whites of the eyes may become yellow, and the urine may become dark, i.e. jaundice.

As the cancer grows and spreads, pain often develops in the upper abdomen and sometimes spreads to the back. The pain may become worse after the person eats or lies down. Cancer of the pancreas can also cause nausea, loss of appetite, weight loss and weakness.

Islet cell cancer can cause the pancreas to make too much insulin or other hormones. When this happens, the person may feel weak or dizzy and may have chills, muscle spasms, or diarrhoea.

The progression of the pancreas is difficult to control. This disease can currently be cured only if diagnosed at an early stage. Cancer that begins in the pancreatic ducts may be treated with surgery, radiation therapy, or chemotherapy or a combination. Islet cell cancer is usually treated with surgery or chemotherapy. A total pancreatectomy, removing the entire pancreas as well as the duodenum, common bile duct, gallbladder, spleen, and nearby lymph nodes, may be necessary.

Pain is a common problem, only partially alleviated by pain killers, or other treatments, such as injecting alcohol into the area around nerves to block the pain, or cutting the nerves in the abdomen during surgery. Cancer of the pancreas and its treatment may interfere with production of pancreatic enzymes and insulin. As a result, patients may have problems digesting food and maintaining the proper blood sugar level.

The reasons for the high frequency of perineural invasion and the presence of the pain in pancreatic cancer are not clear. NGF is involved in stimulating epithelial cancer cell growth and perineural invasion as well as in pain generation in chronic benign disorders. NGF and TrkA have been examined by Northern blot analysis, *in situ* hybridisation and immunocytochemistry in normal and pancreatic tissue samples (Zhu, ZW, *et al* (1999) Journal Of Clinical Oncology Vol.17, No.8, pp.2419-2428). Northern blot analysis showed that NGF and TrkA mRNA levels were significantly increased in pancreatic cancer tissues. *In situ* hybridisation and immunocytochemistry showed a strong presence of NGF in the cytoplasm of pancreatic cancer cells and TrkA was intensely present in the perineurium of pancreatic nerves. It has also been shown that levels of endogenous NGF in pancreatic cancer correlates with degree of perineural invasion and pain. Thus enhanced expression of the NGF/TrkA system may influence perineural invasion (Zhu, ZW, *et al* (1999) Journal Of Clinical Oncology Vol.17, No.8, pp.2419-2428).

International patent application WO 99/11291 discloses a method of treating human brain tumor cells comprising transfecting the cells with a gene encoding the full TrkA receptor. NGF is added and leads to the death of the transfected cells. This disclosure is clearly different from the present invention because cells are transfected with a gene encoding the full TrkA receptor and because it is necessary to add NGF.

The inventors have unexpectedly shown that the growth rate of at least two pancreatic cancer cell lines is inhibited by the presence of TrkAIg2 and at certain higher concentrations cell death is induced.

The inventors have unexpectedly discovered that TrkAIg2 is capable of inhibition of cancer cell growth and mediates cell death.

Accordingly, a first aspect of the present invention provides the use of TrkAIg2 or an analogue thereof in the preparation of a medicament for the treatment and/or prevention of a cancer in a patient.

A second aspect of the invention provides a method of treatment and/or prevention of cancer in a patient, the method comprising supplying to the patient a composition comprising TrkAIg2 or an analogue thereof.

The composition may be supplied for example by ingestion, intravenous injection, intradermal, intraperitoneal, intracerebroventricular or by direct application to the tumour site.

A third aspect of the invention provides a pharmaceutical composition for the treatment and/or prevention of cancer in a patient, the pharmaceutical composition comprising TrkAIg2 or an analogue thereof and a pharmaceutically acceptable carrier, adjuvant or vehicle.

In all the previous aspects of the invention, the cancer may be pancreatic cancer, or may be selected from other cancers, such as, breast cancer, prostate cancer, brain tumours such as glioblastoma, neuroblastoma, skin cancer and lung cancer. Preferably the cancer is pancreatic cancer.

A fourth aspect of the invention provides a method of inhibiting tumour cell growth, the method comprising contacting cells with TrkAIg2 or an analogue thereof.

The term "TrkAIg2" as used herein means the Ig-like sub-domain 2, preferably with the proline rich sequence, which is shown as amino acids 22 to 150 in Figure 2. Preferably TrkAIg2 also includes a 6 x His tag. It is particularly preferred that TrkAIg2 includes the flanking sequence from vector pET15b, which comprises a 6 x His tag, and is shown as amino acids 1 to 21 in Figure 2.

The term "analogue" used in relation to TrkAIg2 refers to functional portions and derivatives of the natural TrkAIg2 sequence. The functional portions and derivatives must retain the function of the full TrkAIg2 sequence, i.e. they must be capable of preventing the growth of cancer cells. Methods for testing the function of portions and derivatives of TrkAIg2 are described in the examples below. An examples of a derivative of TrkAIg2 is the splice variant of TrkAIg2, which does not have the the 6 amino acid insert underlined in Figure 2 (amino acids 130 to 135). The splice variant of TrkAIg2 (i.e. without the 6 amino acid insert) is normally associated with neurons rather than mast or non-neuronal cells. Derivatives of TrkAIg2 includes sequences from other biological sources such as mammals, birds (for example chicken), insects, reptiles or amphibian. Derivatives include variants of the foregoing sequences as a result of the degeneracy of the genetic code and insertion, deletion and substitution variants. Preferably the derivatives have a homology of at least 80%, more preferably at least 90% and most preferably at least 95% to the TrkAIg2 sequence shown in Figure 2. Homology is preferably determined using BLAST. Preferably the derivatives differ by only 1 to 10 amino acids from the sequence of TrkAIg2 given in Figure 2. It is further preferred that any amino acid changes are conservative. Conservative changes are those that replace one amino acid with one from a family of amino acids which are related in their side chains. For example, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar conservative replacement of an amino acid with a structurally related amino acid will not have a major effect on the biological activity of the protein. Mutations which increase the number of amino acids which are capable of forming disulphide bonds with other amino acids in the protein can also be made in order to increase the stability of the protein. Other mutations which increase the desired function of the protein can also be made.

Pharmaceutical compositions of this invention comprise TrkAIg2 or an analogue thereof, with any pharmaceutically acceptable carrier, adjuvant or vehicle. Pharmaceutically acceptable carriers, adjuvants and vehicles that may be used in the pharmaceutical compositions of this invention include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride

mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene- polyoxypropylene-block polymers, polyethylene glycol and wool fat.

The pharmaceutical compositions may be in the form of a sterile injectable preparation, for example, as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to techniques known in the art using suitable dispersing or wetting agents (such as, for example, Tween 80) and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are mannitol, water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or diglycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant such as Ph. Helv or a similar alcohol.

Embodiments of the invention will now be described, by way of example only, and with reference to the accompanying figures in which:

Figure 1 shows schematically the structure of TrkA;

Figure 2 show the nucleotide and amino acid sequence of TrkAIg2 with a 6 His tag.

Figure 3 is a graph showing the reduction in cell metabolic rate with increasing concentrations of TrkAIg2 in the Mia Pa Ca2 pancreatic cancer cell line;

Figure 4 is a photomicrograph of Mia Pa Ca2 pancreatic cancer cell line without (a) and with (b) TrkAIg2 showing dramatic cell death;

Figure 5 shows the effect of addition of TrkAIg2 to human pancreatic cell line PANC-1 on cell viability after [A] 24 hours [B] 48 hours [C] 72 hours [D] 96 hours incubation. The negative control is taken as the metabolic activity of cells at time 0 hours;

Figure 6 is a photomicrograph of Mia Pa Ca2 cells stained with an antibody to TrkAIg2 [mnuA2]; and

Figure 7 is a photomicrograph of staining with antibody to p75 receptor [p75NGFR Me20.4].[a] A875 cells which express large quantities of p75NGFR [b] Mia Pa Ca2 cells.

EXAMPLE 1

Inhibitory action of TrkAIg2 on pancreatic cancer cells

1. Pancreatic cancer cell line MIA-Pa-Ca-2 (ECACC No. 85062806)

TrkAIg2 was prepared as described in WO 99/53055.

Human pancreatic cancer cell line MIA-Pa-Ca-2 ECACC No. 85062806 (European Collection, Porton Down)

The cells were established from tumour tissue of the pancreas of a 65 year old male Caucasian. The cells can be cloned in soft agar and are sensitive to asparaginase, and when taken at passage number 135 have epithelial morphology.

Cells were taken from liquid nitrogen, thawed at 37°C , and maintained in culture for 3 weeks. MIA-Pa-Ca-2 cells were detached and resuspended in 2x DMEM, 20% FCS,

penicillin/streptomycin and 100µl plated out at a density of 4 x 10^3 cells/well in a 96 well plate. Serial dilutions (1:2) of TrkAIg2 were made in sodium phosphate 20mM, sodium chloride 100mM, pH7.4 and an equal volume was immediately added at a range of final concentration of 3.25uM to 0.01uM. A 'buffer only' control was included. After 48 hours without refeeding cell metabolic activity was determined using Promega's CellTitre 96® cell proliferation assay.

The CellTiter 96® Assay is a non-radioactive, colorimetric assay for measuring metabolic activity of viable cells. The assay is composed of solutions of (3-(4,5-dimethylthiazol-2-yl) -5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS) and an electron coupling reagent (phenazine methosulfate; PMS). MTS (Owen's reagent) is bioreduced by cells into a formazan that is soluble in tissue culture medium. The conversion of MTS into the aqueous soluble formazan is accomplished by dehydrogenase enzymes found in metabolically active cells. The quantity of formazan product as measured by the amount of 490nm absorbance is proportional to the number of living cells.

The results are shown in Fig. 3 which is a graph of the observed reduction in metabolic rate with increasing concentrations of TrkAIg2 (µM). Duplicate wells were visualized using an inverted phase microscope. Fig 4A shows Mia Pa Ca2 cells without the presence of Ig2.

2. Pancreatic cancer cell line PANC1 ECACC No. 87092802

The cells were established from ductal tumour tissue of the pancreas of a 56 year old male Caucasian.

Cells were taken from liquid nitrogen, thawed at 37°C, and maintained in culture for 3 weeks. MIA-PA-CA-2 cells were detached and resuspended in 2x DMEM, 20% FCS, penicillin/streptomycin and 100µl plated out at a density of 5 x 10^3 cells/well in a 96 well plate. Serial dilutions (1:2) of TrkAIg2 were made in sodium phosphate 20mM, sodium chloride 100mM, pH7.4 and an equal volume was diluted in 2xDMEM serum free medium 1:1 added at a range of final concentration of 4.7µM to 0.02µM to start concentration in 1xDMEM medium.

Mia Pa Ca2 cells were grown on Essex-Henley slides and incubated with antibodies to TrkAIg2 (mnuA2) or p75 (HB8737 me20.4) followed by anti-rabbit IgG-FITC conjugated or anti-mouse FITC conjugated respectively. Cells were visualized using a Leitz microscope with fluorescence module. Cells were shown to express TrkA receptors (Fig. 6) but not p75 (Fig.7b). By contrast, a positive control, A875 cells which express p75NGFR receptors did stain with this antibody (Fig. 7a).

The results are shown in Fig. 5 from 4.7 to 0.03uM TrkAIg2 caused cell death and from 0.30 to 0.02μM, it inhibited cell growth.

Studies suggest that other types of cancer may also rely on the presence of NGF for growth and proliferation (Sortino, M. A. *et al* (2000) *Molecular Endocrinology* Vol. 14 **(No. 1)**: 124-136; Walch, E. T. *et al* (1999) *Clinical & Experimental Metastasis* ***Vol. 17 (No.4)***: 307-314; Descamps, S. *et al* (1998) *Journal of Biological Chemistry* Vol. 273 **(No. 27)**: 16659-16662)

Prostate Cancer

NGF may play a role in some prostate cancers. Studies on the androgen-dependent, prostate adenocarcinoma LNCaP cell line (Sortino, M. A. *et al* (2000) *Molecular Endocrinology* Vol. 14 (No. 1): 124-136) show that application of NGF results in a concentration-dependent increase in proliferation. This is accompanied by an enhanced expression of prostate-specific antigen (PSA) and added to the proliferative effect of dihydrotestosterone. The proliferative effect of NGF appeared to be mediated by TrkA. TrkA but not p75(NGFR) was expressed in LNCaP cells; but both the proliferative response and the phosphorylation of TrkA upon NGF treatment were prevented by the tyrosine kinase inhibitor K252a. LNCaP cells transiently transfected with the cDNA encoding for p75(NGFR) appeared more sensitive to NGF, and increased in number when exposed for 72 h to NGF compared with wild LNCaP cultures.

Furthermore, Walch *et al.*, using this same human prostate cancer cell line (LNCaP), and also PC-3, and DU145, demonstrate that NGF and NT-4 increase *in vitro* invasion (Walch, E. T. *et al* (1999) *Clinical & Experimental Metastasis* ***Vol. 17 (No.4)***: 307-314). In addition the expression of heparanase, a molecular determinant of tumour metastasis, was found to

be induced. The effects were most marked in the DU145 cells. It is reported that these lines had negligible TrkA and TrkC expression, although TrkB was expressed in all three prostatic tumour cell lines examined. The DU145 cells were also positive for p75(NGFR). The study showed that NGF and NT4 are important in metastasis and that their expression coincides with transformation to a malignant phenotype capable of invasion along the perineural space and extracapsular metastasis to distant sites.

These facts make it highly likely that certain prostate tumours may respond well to treatment with TrkAIg2 which will sequester endogenous NGF.

Breast Cancer

There also seems to be good evidence to support a role of NGF in breast cancer. Descamps and colleagues (Descamps, S. *et al* (1998) *Journal of Biological Chemistry* Vol. 273 **(No. 27)**: 16659-16662) show that NGF is able to stimulate the proliferation of breast cancer cells (MCF-7 and MDA-MB-231 cell lines), although it is unable to stimulate growth of normal breast epithelial cells. This abnormal stimulation induces cells in the G(0) phase to re-enter the cell cycle, as well as shortening cell cycle duration. The two cancer cell lines and the normal breast cell line express TrkA and p75(NGFR) receptors. Activation of mitogen-activated protein kinase can be detected in breast cancer cells after 10 min of NGF stimulation, whereas no change was detected in normal breast cells.

Of course this may not be true of all breast cancer cell lines. However, Tagliabue *et al.* show TrkA mRNA in 12 of 14 human breast carcinoma specimens and three of four cell lines (Tagliabue, E. *et al* (2000) *Journal of Biological Chemistry* Vol. 275 **(No. 8)**: *5388-5394)*. NGF stimulated two of the three TrkA-expressing cell lines. Importantly, inhibition of NGF-induced activation by an antibody directed against the extracellular domain of TrkA (but not by an inhibitor of only TrkA phosphorylation) demonstrated the requirement of NGF binding but not of TrkA kinase activity of MAPK activation, suggesting that recruitment of another kinase for transmission of the mitogenic signalling. This means that in order to stop the NGF-induced stimulation in these cells, it is necessary to remove or inhibit the effect of NGF on the extracellular region of the TrkA receptor.

It seems likely therefore that treatment with TrkAIg2 will be of benefit to patients with breast cancer.

Brain Tumour

Metastatic tumour cells in the brain which attach to endothelial cells and respond to brain-derived invasion factors, can invade the blood-brain barrier. In responsive tumour cells, neurotrophins promote invasion by enhancing the production of basement-membrane-degradative enzymes, such as gelatinase and heparanase, which cause a local breakdown of the blood-brain barrier. Menter and colleagues (Menter D. G. *et al* (1994) *Involvement of Neurotrophins and Growth-Factors in Brain Metastasis Formation Invasion & Metastasis Vol. 14* **(No. 1-6)**: 372-384) found increased levels of NGF in tumour-adjacent tissues at the invasion front of human melanoma tumours in the brain. In addition, the proliferation of a glioblastoma cell line (87 HG 31) could be stimulated by NGF (Delman N *et al* (1995) Cancer Research Vol. 55 (No. 10): 2212-2219). The addition of TrkAIg2 to these tissues is expected to result in a decrease in tumour proliferation.

Lung Cancer

Clonal growth of three lung cancer cell lines (HTB 119, HTB 120, CCL 185) could be stimulated up to 3-fold by NGF with a dose-response relationship (0.5-500 ng/ml) (Oelmann, E. *et al* (1995) *Cancer Research* Vol. 55 **(No. 10)**: 2212-2219). This effect was completely reversible by anti-NGF antibody and by the tyrosine kinase inhibitor genistein.

Epithelial Cancer

NGF has been suggested to be a potent regulator of cell proliferation in human epithelial cells (Di Marco, *et al* (1993). *Journal Of Biological Chemistry*, 268, 22838- 22846). Normal human keratinocytes synthesize and secrete biologically active NGF in a growth regulated fashion (DiMarco E., *et al* (1991) *Journal Of Biological Chemistry* 266, 21718-21722). Keratinocytes express both the low (p75(NGFR)- and the high-affinity (TrkA) NGF- receptors. NGF upregulates the expression of NGF mRNA (Pincelli, C. and Marconi, A. (2000) *Journal of Dermatological Science Vol. 22 (No. 2): 71-79)*. K252, which inhibits trk phosphorylation, blocks NGF-induced keratinocyte proliferation and induces apoptosis in normal keratinocytes. Furthermore, normal keratinocytes

over-expressing either TrkA or NGF proliferate better than controls (Pincelli, C. and Marconi, A. (2000) *supra)*.

Therefore, in view of the previous NGF studies carried out by the inventors, it is possible that NGF is involved in cell proliferation, particularly with reference to tumourous cells. It seems likely that in many of these cell types the addition of the NGF sequestering agent TrkAIg2 will inhibit proliferation and may, as in pancreatic tumour cell lines, cause actual cell death on application. However, this theory has never previously been expressed or tested.

All documents referred above are incorporated herein by reference.

Claims

1. Use of TrkAIg2 or an analogue thereof in the preparation of a medicament for the treatment and/or prevention of cancer in a patient.

2. A method of treatment and/or prevention of cancer in a patient comprising supplying to the patient a composition comprising TrkAIg2 or an analogue thereof.

3. A method according to claim 2 wherein the TrkAIg2 or analogue is supplied by ingestion, intravenous injection, intradermal, intraperitoneal, intracerebroventricular or by direct application to the tumour site.

4. A method of inhibiting tumour cell growth, the method comprising contacting cells with TrkAIg2 or an analogue thereof.

5. A pharmaceutical composition for the treatment and/or prevention of cancer in a patient, the pharmaceutical composition comprising TrkAIg2 or an analogue thereof in combination with a pharmaceutically acceptable carrier, adjuvant or vehicle.

6. Use of TrkAIg2 or an analogue thereof according to claim 1, or a method according to claim 2 or claim 3, or a pharmaceutical composition according to claim 4, wherein the cancer is pancreatic cancer.

7. Use of TrkAIg2 or an analogue thereof according to claim 1, or a method according to claim 2 or claim 3, or a pharmaceutical composition according to claim 4, wherein the cancer is selected from breast cancer, prostate cancer, brain tumours, skin cancer or lung cancer.

FIG. 1

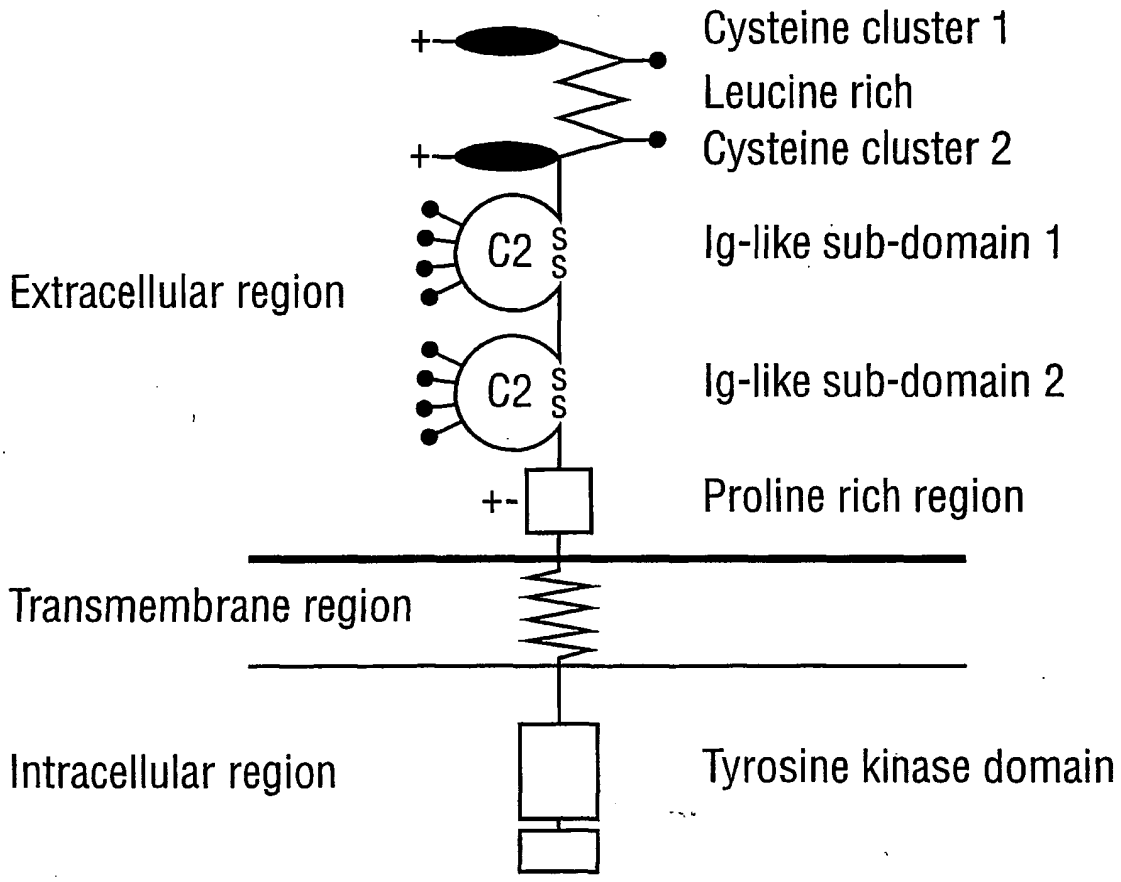

Cysteine cluster 1
Leucine rich
Cysteine cluster 2
Ig-like sub-domain 1
Ig-like sub-domain 2
Proline rich region
Extracellular region
Transmembrane region
Intracellular region
Tyrosine kinase domain
C2
S
S
C2
S
S
+
+
+-

FIG. 2

```
    Met Gly Ser Ser His His His His His His Ser Ser      12
  1 ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC AGC
    Gly Leu Val Pro Arg Gly Ser His Met Pro Ala Ser      24
 37 GGC CTG GTG CCG CGC GGC AGC CAT ATG CCG GCC AGT
    Val Gln Leu His Thr Ala Val Glu Met His His Trp      36
 73 GTG CAG CTG CAC ACG GCG GTG GAG ATG CAC CAC TGG
    Cys Ile Pro Phe Ser Val Asp Gly Gln Pro Ala Pro      48
109 TGC ATC CCC TTC TCT GTG GAT GGG CAG CCG GCA CCG
    Ser Leu Arg Trp Leu Phe Asn Gly Ser Val Leu Asn      60
145 TCT CTG CGC TGG CTC TTC AAT GGC TCC GTG CTC AAT
    Glu Thr Ser Phe Ile Phe Thr Glu Phe Leu Glu Pro      72
181 GAG ACC AGC TTC ATC TTC ACT GAG TTC CTG GAG CCG
    Ala Ala Asn Glu Thr Val Arg His Gly Cys Leu Arg      84
217 GCA GCC AAT GAG ACC GTG CGG CAC GGG TGT CTG CGC
    Leu Asn Gln Pro Thr His Val Asn Asn Gly Asn Tyr      96
253 CTC AAC CAG CCC ACC CAC GTC AAC AAC GGC AAC TAC
    Thr Leu Leu Ala Ala Asn Pro Phe Gly Gln Ala Ser     108
289 ACG CTG CTG GCT GCC AAC CCC TTC GGC CAG GCC TCC
    Ala Ser Ile Met Ala Ala Phe Met Asp Asn Pro Phe     120
325 GCC TCC ATC ATG GCT GCC TTC ATG GAC AAC CCT TTC
    Glu Phe Asn Pro Glu Asp Pro Ile Pro Val Ser Phe     132
361 GAG TTC AAC CCC GAG GAC CCC ATC CCT GTC TCC TTC
    Ser Pro Val Asp Thr Asn Ser Thr Ser Gly Asp Pro     144
397 TCG CCA GTG GAC ACT AAC AGC ACA TCT GGA GAC CCG
    Val Glu Lys Lys Asp Glu                             150
433 GTG GAG AAG AAG GAC GAA
    Stop
452 TGA TAACTCGAGATCGG
```

FIG. 3

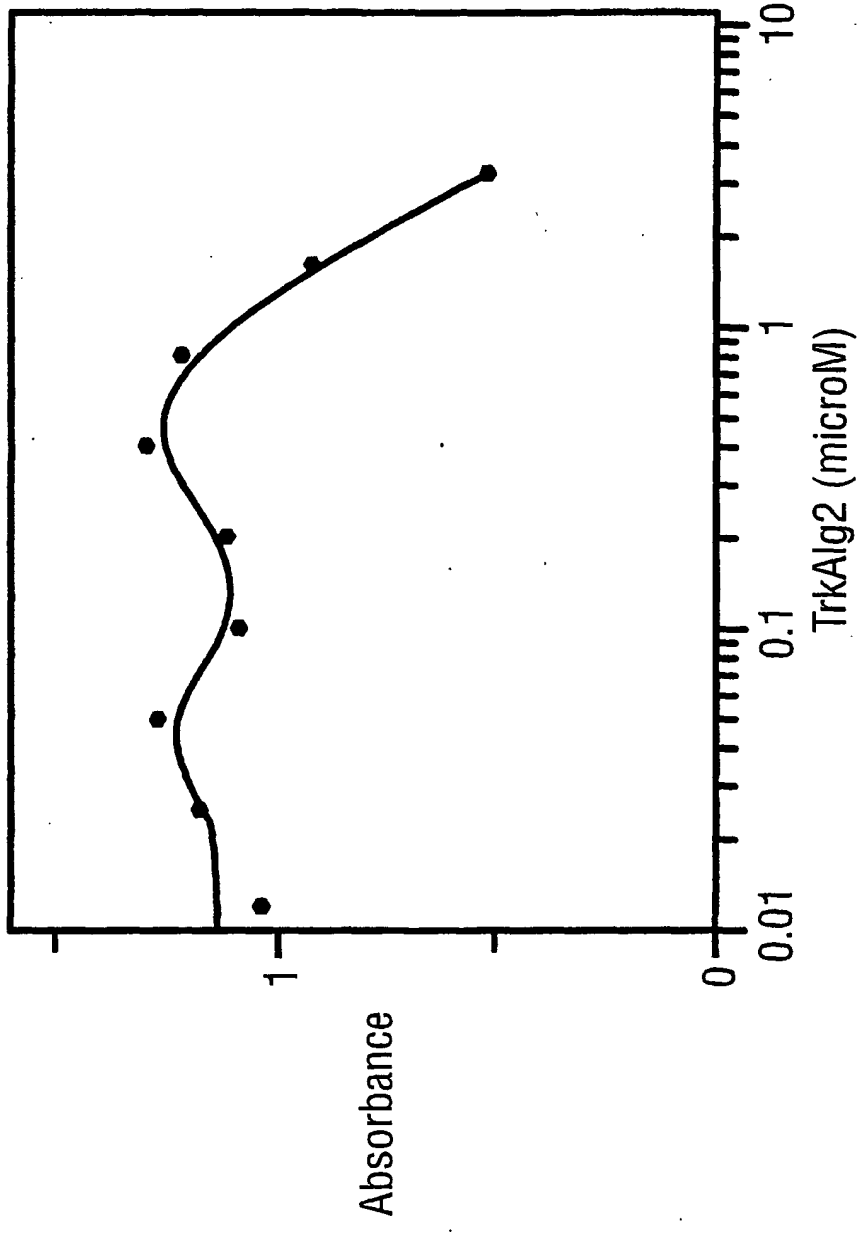


FIG. 5

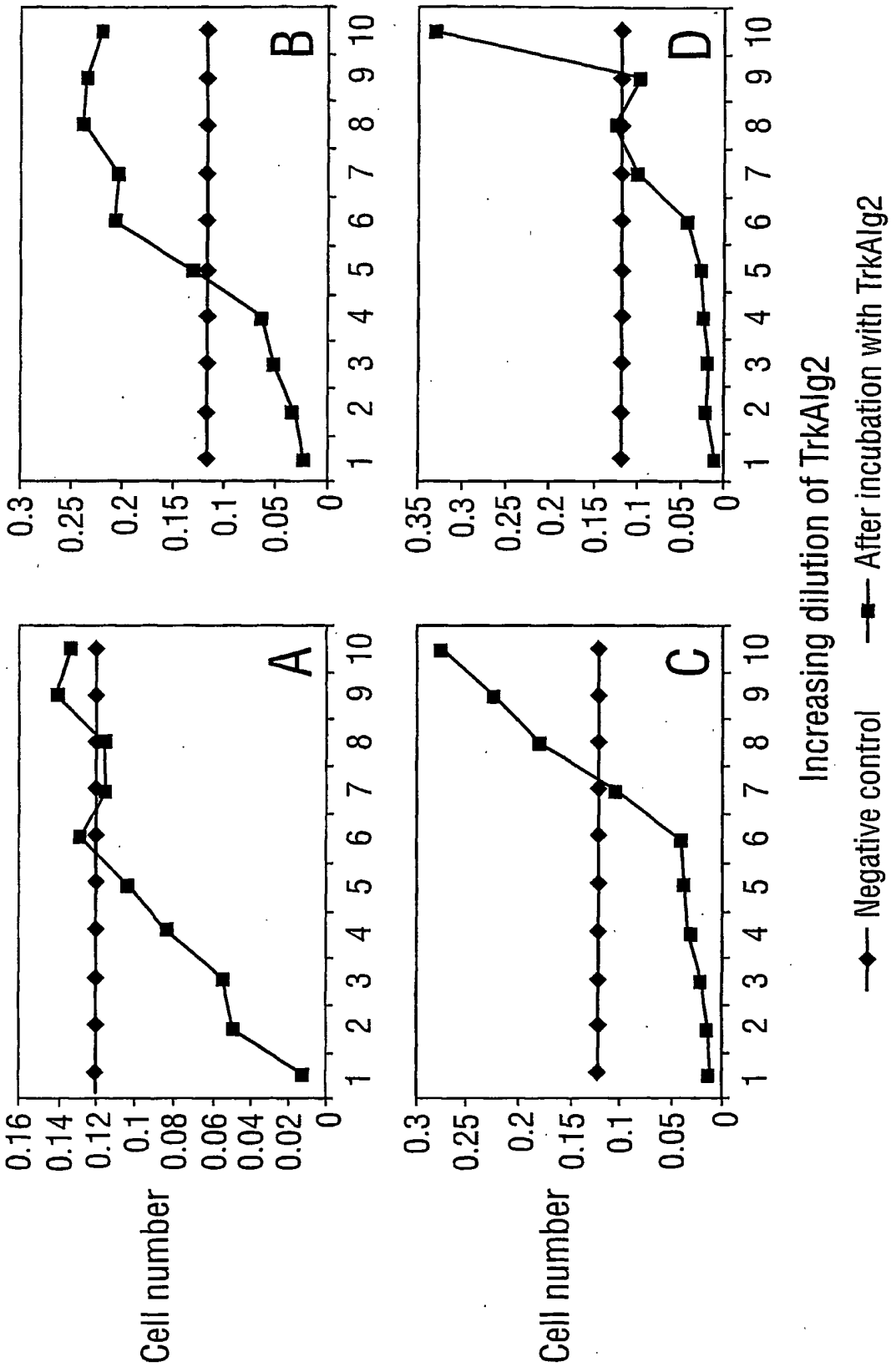
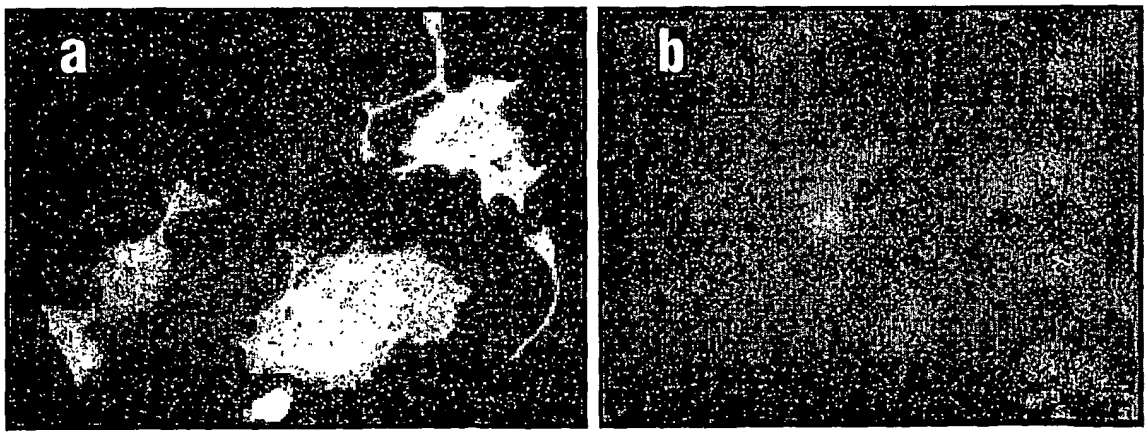

A
B
C
D
Cell number
Cell number
Increasing dilution of TrkAlg2
Negative control
After incubation with TrkAlg2

FIG. 6

FIG. 7



INTERNATIONAL SEARCH REPORT

Inte nal Application No
PCT/GB 01/03682

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/17 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EMBASE, CHEM ABS Data, WPI Data, PAJ, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 99 53055 A (UNIVERSITY OF BRISTOL) 21 October 1999 (1999-10-21) cited in the application the whole document	1-7
Y	WO 95 25795 A (GENENTECH, INC.) 28 September 1995 (1995-09-28) examples 3,5 page 53, line 11	1-7
Y	WO 96 09387 A (WORCESTER FOUNDATION FOR BIOMEDICAL RESEARCH) 28 March 1996 (1996-03-28) examples claims	1-7

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 November 2001	13/12/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Nooij, F

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	A. ROBERTSON ET AL.: "Structure of TrkAIg2 and molecular modelling of the neurotrophin binding sites of the Trk receptors." EUROPEAN JOURNAL OF NEUROSCIENCE, vol. 12, no. suppl. 11, 27 June 2000 (2000-06-27), page 329 XP002184446 Oxford, GB abstract 155.06	1-7
A	V. ASOPA ET AL.: "Expression, purification, refolding and characterisation of the immunoglobulin-like sub-domains of the nerve growth factor receptor (TrkA)." BRITISH JOURNAL OF PHARMACOLOGY, vol. 119, no. Proc. Suppl., October 1996 (1996-10), XP001030926 London, GB abstract 273P	1-7
A	L. O'CONNELL ET AL.: "TrkA amino acids controlling specificity for nerve growth factor." THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 275, no. 11, 17 March 2000 (2000-03-17), pages 7870-7877, XP002184445 Baltimore, MD, USA abstract	1-7
A	P. HOLDEN ET AL.: "Immunoglobulin-like domains define the nerve growth factor binding site of the TrkA receptor." NATURE BIOTECHNOLOGY, vol. 15, no. 7, July 1997 (1997-07), pages 668-672, XP002116514 New York, NY, USA cited in the application the whole document	1-7

INTERNATIONAL SEARCH REPORT

information on patent family members

Inte[illegible]nal Application No
PCT/GB 01/03682

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9953055 A	21-10-1999	AU 3433699 A	01-11-1999
		EP 1068315 A2	17-01-2001
		WO 9953055 A2	21-10-1999
WO 9525795 A	28-09-1995	US 5877016 A	02-03-1999
		US 5844092 A	01-12-1998
		AU 707613 B2	15-07-1999
		AU 3245695 A	09-10-1995
		AU 5399099 A	02-12-1999
		CA 2184059 A1	28-09-1995
		EP 0750666 A1	02-01-1997
		JP 9510612 T	28-10-1997
		NZ 300064 A	26-08-1998
		WO 9525795 A1	28-09-1995
		US 6153189 A	28-11-2000
		US 6025166 A	15-02-2000
		US 5910574 A	08-06-1999
		US 6027927 A	22-02-2000
WO 9609387 A	28-03-1996	US 5789187 A	04-08-1998
		AU 3637595 A	09-04-1996
		CA 2200611 A1	28-03-1996
		EP 0784679 A1	23-07-1997
		JP 2000510321 T	15-08-2000
		WO 9609387 A1	28-03-1996
		US 6271205 B1	07-08-2001